

THERMAL-SHOCK TOLERANCE OF THREE SPECIES OF AQUATIC INSECTS IN A NORTHERN CALIFORNIA, GEOTHERMALLY INFLUENCED STREAM

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Abstract.—This study assessed the response of aquatic insects to short-term thermal shocks, such as those encountered upon drifting into heated areas of geothermally influenced streams. The upper incipient lethal temperature (UILT) thresholds were determined for three common species in Big Sulphur Creek, Sonoma County, California, and the effect of acclimation temperature on the UILT was determined for two caddisflies, both of which overwinter in the stream as larvae.

When acclimated at 28° C, the LT_{50} of *Centroptilum convexum* (Ide) ranged from 36.3° to 38.5°, that of *Gumaga nigricula* (McLachlan) from 37.8° to 41.0°, and that of *Helicopsyche borealis* (Hagen) from 38.5° to 41.4°. When acclimated at 5.5°, the LT_{50} for *G. nigricula* ranged from 35.7° to 38.9° and that of *H. borealis* from 36.6° to 41.1°. *Helicopsyche borealis* has the highest UILT of any caddisfly, 39.0° for 30 min. The most important factors determining the UILTs in these insects are acclimation temperature, shock duration, and the maximum temperature of exposure.

Key Words.—Insecta, Trichoptera, Ephemeroptera, thermal-shock, geothermal, bioassay, thermal tolerance

Aquatic insects inhabiting streams in geothermal areas are often subject to high water temperatures. Insects that drift in these streams may encounter elevated temperatures for brief periods, whereas those in benthic communities downstream from thermal inputs (e.g., hot springs) generally experience longer exposures, particularly during periods of reduced streamflow. The severity of stress on an insect in such conditions depends upon several variables, including: 1) duration and magnitude of temperature change; 2) the maximum temperature experienced; 3) acclimation temperature; and 4) body size, life stage, and physiological condition of the organism (Hutchison 1976).

Most studies of thermal tolerance of aquatic insects have examined either short-term (i.e., exposures of up to several days) or chronic (i.e., exposure throughout the immature stages) effects of heated discharges. Short-term effects are typically determined by a standard bioassay using either 24 to 96 h exposure times (e.g., Nebeker & Lemke 1968, Gaufin & Hern 1971, deKozlowski & Bunting 1981) or the Critical Thermal Maximum (CTM) procedure (e.g., Moulton et al. 1993). For CTM the animals are heated at a constant rate (usually 0.5° C per min) until locomotory activities become disorganized (Ernst et al. 1984). Chronic effects have been studied using a number of methods (e.g., Cairns 1976, Gartman & Lake 1979). The effect of rapid immersion, called short-term thermal shock, has received much less attention (Sherberger et al. 1977, Salmela & Anderson 1978). A short-term bioassay is more appropriate than the 24 or 96 h or CTM test in some cases, such as determining the tolerance of insects drifting into heated discharges. Exposure times during entrainment in power plant cooling systems, for

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example, seldom exceed 1 h (Schubel et al. 1978). These authors argue that when exposure times are brief, rapid exposure to test temperatures is better than the gradual increases of the critical thermal maximum (CTM) method.

Naturally heated waters present a unique opportunity for investigating the influence of thermal gradients on structural and functional aspects of benthic communities. Consequently, biological communities in geothermal waters have been well studied (e.g., Lamberti & Resh 1985, Resh & Barnby 1987 and references therein). The potential that such waters offer for field experiments has been noted by Brock (1970, 1975) but only a few investigators have used these habitats for this purpose (e.g., Pritchard & Pelchat 1977, Schott & Brusven 1980, Lamberti & Resh 1983). Although lethal temperature limits have been established for some aquatic insects exposed to power plant discharges (e.g., Garten & Gentry 1976, Tennessen & Miller 1983), little is known about thermal tolerance of insects that typically occur in geothermal waters.

This study assessed the response of three stream insects to short-term thermal shocks in the geothermal waters of Big Sulphur Creek, The Geysers, Sonoma County, California. Specifically we ask: 1) What are the Upper Incipient Lethal Temperature (UILT) thresholds for these insects? 2) What effect does acclimation temperature have on the UILT? and 3) Does the magnitude of the temperature change (ΔT , i.e., the difference between the acclimation temperature and the lowest LT_{50} observed) during a thermal shock alter the UILT?

MATERIALS AND METHODS

Study area.—We conducted this research in Big Sulphur Creek (BSC), Sonoma County, California, USA ($38^{\circ} 46' N$, $122^{\circ} 45' W$, elevation 680 m), a third-order stream that flows northwesterly through The Geysers Known Geothermal Resources Area. The Geysers is the largest geothermal electric power-producing facility in the world, but natural surface expressions are confined to hot springs and a few steam vents. Our experiments were conducted approximately 250 m below the confluence of BSC and its tributary Little Geysers Creek (LGC). The lower portion of LGC is heated by a series of hot springs. The waters of LGC cool rapidly when they mix into BSC, which is not affected by geothermal inputs upstream of LGC. The thermal influence of LGC is not detectable after the first 200 m of mixing in BSC (Lamberti & Resh 1985). For a more detailed description of this site including water chemistry, thermal, and geological conditions see Lamberti & Resh (1983), McColl et al. (1978), McMillan (1985) and McElravy et al. (1989).

Thermal-shock bioassays were conducted in BSC on 17 Jul 1982 ($28^{\circ} C$ acclimation temperature) and 14 Jan 1983 ($5.5^{\circ} C$ acclimation temperature). Immature stages of three species of aquatic insects common in BSC, the caddisflies *Helicopsyche borealis* (Hagen) and *Gumaga nigricula* (McLachlan) and the mayfly *Centroptilum convexum* (Ide) were tested in July; *C. convexum*, however, was not present in sufficient numbers for winter testing.

Design of heat shock experiment.—All tests were conducted at streamside, eliminating the need for the 1–3 week pre-test holding period usually required in laboratory-based bioassays, and reducing many non-thermal stresses (e.g., handling, transportation). Additionally, this procedure allowed the test-cell environ-



Figure 1. Floating racks used to hold test cells and monitor effects of thermal-shock on stream macroinvertebrates in Big Sulphur Creek, The Geysers, Sonoma Co., California.

ment to fluctuate with ambient conditions during holding periods and provided a more realistic bioassay.

Test insects were collected at the study site and placed in groups of ten into each of 42 PVC test cells (5 cm diameter with screened bottoms) supported in the stream by a floating platform. Test cells were arranged in a 6×7 time-temperature matrix described below. Three replicates of cells were prepared and supported in BSC by a floating platform (Fig. 1) for <12 h prior to testing. For each species a total of 1260 insects were tested in the three replicate experiments.

Seven controlled-temperature water baths in portable ice chests ($20 \times 50 \times 20$ cm) were set up next to the stream. Submersible aquarium heaters were used in each bath to maintain constant temperature, and an air pump provided aeration and mixing. Water temperatures were monitored manually and fluctuations in the baths were kept to $<0.5^\circ$ C by hand additions of warm water when necessary. Results of preliminary tests were used to establish a site-specific time-temperature matrix that would bracket from 0 to 100% mortality. In setting time levels for this matrix, we considered the minimum possible drift times (determined with a dye tracer) through thermal portions of LGC to the confluence with the cooler waters of BSC. The six test-temperature baths were 33° , 36° , 39° , 41° , 43° and 45° C; the control bath was held at ambient stream temperature. Six exposure times were used (5, 10, 15, 20, 30, and 60 min) at each temperature.

Experimental procedure.—The thermal shocks were administered by quickly transferring the test cells from the platform in BSC to the pre-heated water baths on shore, and immediately immersing them. After the appropriate time interval

the cells were removed from the bath, returned to the platform, and percent mortality determined after 24 h. Individuals were considered dead when they failed to respond to repeated probing. The median resistance temperature (LT_{50}) for each exposure time was determined graphically following the method of Litchfield & Wilcoxon (1949). The upper incipient lethal temperature (UILT) was computed for each time-temperature combination from the replicate LT_{50} values (Hutchison 1976, Schubel et al. 1978). Exposure times (min) were log-transformed [$\text{Log}_{10}(X_i + 1)$] to correct for non-linearity (Zar 1974). Simple linear regression of median LT_{50} temperature against exposure time was used to determine the UILT equations. Two-way ANOVA (Zar 1974) was used to compare the median LT_{50} 's among species and acclimation temperatures.

RESULTS AND DISCUSSION

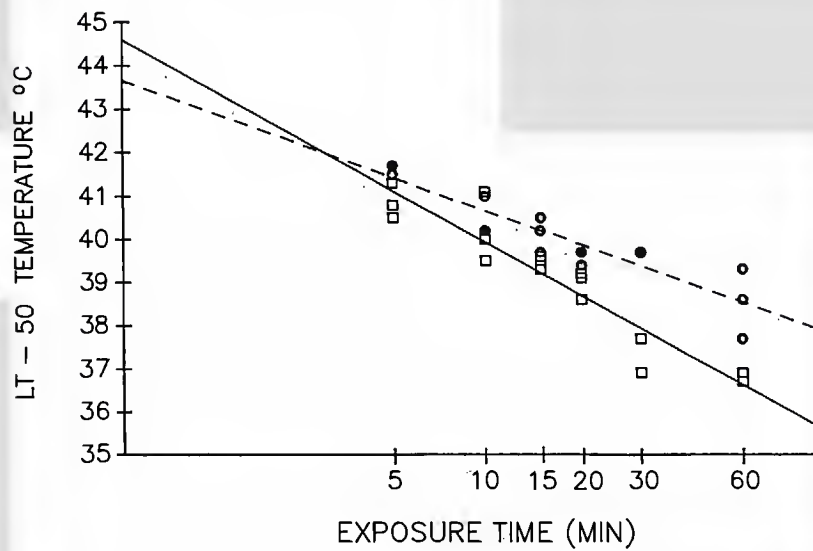
Helicopsyche borealis.—When acclimated at 28° C, this caddisfly has the highest 1 h resistance to thermal shock among the three species tested ($LT_{50} = 38.5^\circ$ C). It endured a 1 h exposure at 36° C in both summer and winter with no mortality. The UILT equations of *H. borealis* for July and January were significantly different (ANOVA, $P < 0.0005$). The 5 and 60 min LT_{50} temperatures estimated by the UILT equations ranged from 41.4 to 38.5° C in summer and 41.1 to 36.6° C in winter (Fig. 2). This species' resistance to thermal shock is slightly lower in winter (1–2° C) than in summer, as indicated by the greater negative slope of the winter UILT equation, especially at the longer durations tested. Previous reports have set the maximum thermal tolerance for *H. borealis* at 34° C, but these were based on field observations (Wiggins 1977), not bioassays. If the exposure time is limited to less than 30 min, *H. borealis* can survive temperatures as high as 39° C. This is a higher temperature tolerance than that reported for any other caddisfly (Moulton et al. 1993). It is not surprising then that *H. borealis* is widespread across North America (Resh et al. 1984) and that it tolerates relatively low oxygen levels (Williams et al. 1983).

The ΔT experienced by *H. borealis* during these tests was 9.7° C in summer and more than three times as large (31.4° C) in winter. Thus, the total temperature change experienced by this species appears to have little effect on its survival threshold.

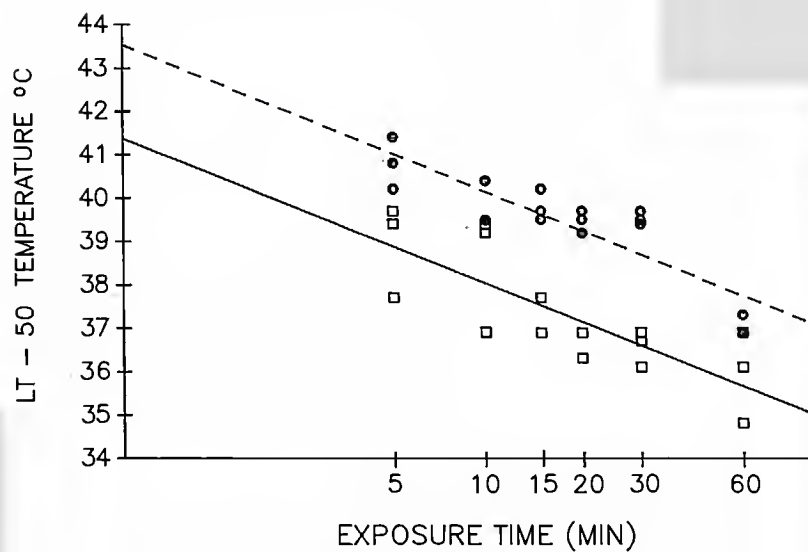
Gumaga nigricula.—When acclimated at 28° C, this caddisfly had a 1 h LT_{50} of 37.8° C. It was able to survive a temperature of 36° C for 1 h during the summer with no mortality, but could not do so in winter. *Gumaga nigricula* has a lower UILT during winter than in summer (ANOVA, $P < 0.0005$). The 5 and 60 min LT_{50} temperatures from the UILT equations ranged from 41 to 37.8° C in summer and 38.9 to 35.7° C in winter (Fig. 3). The ΔT in summer was 8.9° C and it was 29.3° C in winter.

Centroptilum convexum.—The 1 h LT_{50} for *C. convexum* was 36.3° C. However, this mayfly had some mortality at all test conditions, compared with no mortality at 36° C for the two caddisflies when acclimated to 28° C. The summer UILT for this species was significantly different (ANOVA, $P < 0.01$) from either caddisfly, indicating that this mayfly was less tolerant of high temperatures for all durations tested. The LT_{50} temperature for 5 and 60 min ranged from 38.5 to 36.3° C in summer. Mayflies have consistently been shown to be less tolerant of temperature

HELICOPSYCHE BOREALIS



GUMAGA NIGRICULA



CENTROPTILUM CONVEXUM

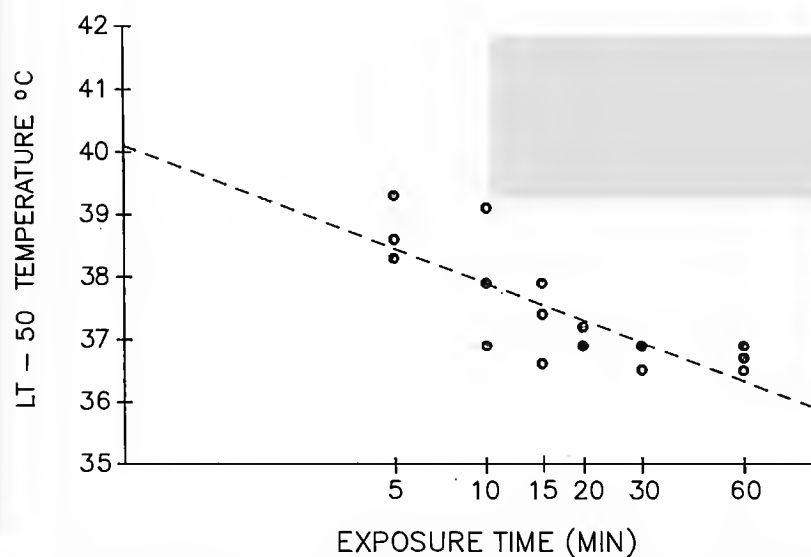


Figure 2-4. LT_{50} thermal-shock values and the associated UILT regression lines at two acclimation temperatures. Designations, summer (28°C) ---●---●---; winter (5.5°C) —□—□—. Figure 2. *Helicopsyche borealis*. Summer UILT equation is $Y = -2.87x + 43.65$; $r^2 = 0.84$, $P < 0.0001$. Winter UILT equation is $Y = -4.47x + 44.59$; $r^2 = 0.88$, $P < 0.0001$. Figure 3. *Gumaga nigricula*. Summer UILT equation is $Y = -3.24x + 43.53$; $r^2 = 0.76$, $P < 0.0001$. Winter UILT equation is $Y = -3.2x + 41.36$; $r^2 = 0.62$, $P = 0.0001$. Figure 4. *Centroptilum convexum*. Summer UILT equation is $Y = -2.11x + 40.1$; $r^2 = 0.61$, $P = 0.0001$.

increases than caddisflies (e.g., Gaufin & Hern 1971, deKozlowski & Bunting 1981, Tennessen & Miller 1983).

The UILT for aquatic insects can be expected to vary with respect to acclimation temperature in three ways (Precht et al. 1973): 1) UILT may be unaffected by acclimation temperature and show no variation; 2) UILT may be positively related to acclimation temperature, thus increasing and decreasing with seasonal temperature fluctuations (this is the most commonly observed relationship in aquatic organisms); or 3) UILT may be negatively related to the acclimation temperature. We found that the UILTs of *H. borealis* and *G. nigricula* are lower in the winter than in the summer and that this seasonal affect is greater for *H. borealis*. This diminished thermal tolerance may be due to the physiological state of this caddisfly. It overwinters in a quiescent state, attached at the base of boulders (Williams et al. 1983, Resh et al. 1984b).

Moulton et al. (1993) found a similar positive correlation with acclimation temperature in *Hydropsyche simulans* Ross, *Ceratopsyche morosa* (Hagen), *Chimarra obscura* (Walker), and *Chimarra aterrima* Hagen. However, their results and ours contrast with those for two other caddisflies, *Hydropsyche* sp. (Sherberger et al. 1977) and *Brachycentrus americanus* (Banks) (Salmela & Anderson 1978), both of which were unaffected by acclimation temperature. The differences may be due, in part, to variations in the experimental protocols. Salmela & Anderson (1978) used fewer time-temperature combinations than in our matrix. Sherberger et al. (1977) lowered the post-shock temperature slowly, whereas we used an instantaneous decrease. We held the insects in the field for a period of only 24 h for post-shock evaluation.

During this study the maximum temperature at the study site in BSC was 30.5° C. In summer, the aquatic insects in this portion of the stream are living near their UILT and a relatively small increase in stream temperature (i.e., a ΔT of 6–9° C) would exceed the UILT determined for all three test species. This was illustrated in a related study of the thermally influenced portion of LGC upstream from our study site. Resh et al. (1984a) found that very large numbers of macroinvertebrates (primarily Chironomidae and Oligochaeta) occurred in the afternoon drift. These animals were probably induced to drift catastrophically as the substrate temperature exceeded their UILTs. In winter, there is a small reduction in the UILT for the two caddisflies. However, since ambient water temperatures are low, and both insects can withstand a $\Delta T > 30^\circ$ C, these species would thus be less susceptible to the effect of thermal discharges at this time of year.

An insect drifting into a thermal hot spring discharge would not be killed by rapid heating, provided the temperature did not exceed its UILT. We conclude, as did Sherberger et al. (1977), that the magnitude of the thermal shock is not consequential in inducing mortality unless it approaches the UILT. The most important factors determining the UILTs in *H. borealis*, *G. nigricula*, and *C. convexum* appear to be acclimation temperature, shock duration, and the maximum temperature of exposure.

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